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Sturgeon Detection Using Biochemical Methods

PURPOSE: This technical note explores the concept of biochemical methodologies to survey waterways for the presence of sturgeon. Such a tool would enhance capabilities to detect sturgeon occupation of channel reaches in advance of dredging operations, and to direct longer-term inventories of habitat use within entire water basins. Coordination and management of navigation dredging projects can become very difficult if navigation channel reaches to be dredged are known to be frequented by sturgeon. Almost all sturgeon stocks are subject to special protective measures based on their status as threatened and/or endangered species on many state or federal registers. Given the general rarity of sturgeon and lack of knowledge regarding their population dynamics, habitat requirements, and seasonal occurrences in many portions of navigable waterways, dredging projects are frequently constrained to avoid detrimental effects.

BACKGROUND: Hypothetical impacts on sturgeon from dredging projects include habitat disturbance via in-water dredged material disposal practices or hydraulic entrainment of early life history stages, including juveniles. Constraints on dredging projects often take the form of environmental windows, which seek to avoid temporal overlap between sturgeon habitat utilization and dredging or dredged material disposal operations (Reine, Dickerson, and Clarke 1998). Presently, few technologies exist which can reliably yield estimates of sturgeon stocks within a given reach of waterway. Conventional fishery survey methods are largely inadequate, particularly during periods of high water current flows, or where sturgeon occasionally congregate in deep holes. Accurate surveys of early life history stages and juveniles have proven to be very difficult to conduct. These considerations stimulated the present investigation of alternative methods to survey navigable waterways for the presence of sturgeon.

Potential conflicts between dredging operations and sturgeon protection have been a concern of resource agencies for decades. A brief summary of relevant studies follows, with no attempt to present a comprehensive treatment of the literature. One of the earliest references to detrimental impacts of dredging on sturgeon was that of Veshchev (1981), who indirectly estimated rates of sturgeon larvae (*Acipenser guldenstaedti* and *A. stellatus*) entrainment as high as 76.8 percent in the Volga River in Russia. This account was anecdotal, however, as details of the dredging operation and estimation methods were not given. Hastings (1983) also noted anecdotal accounts of adult sturgeon "being expelled from dredge spoil pipes" into confined dredged material disposal facilities along the Delaware River. Buell (1992) reported that juvenile white sturgeon (*A. transmontanus*) were incidentally entrained by a hydraulic dredge during excavation of sand from a relatively deep segment of the Columbia River, Oregon. Few other documented cases of sturgeon entrainment exist, although infrequent "takes" of shortnose sturgeon (*A. brevirostrum*) have been recorded by Threatened and Endangered Species Protection Program observers aboard hopper dredges in various Atlantic coast rivers and estuaries.

Aside from entrainment, habitat disruption appears to be the most likely mechanism by which dredging and dredged material disposal operations might affect sturgeons detrimentally. Here too,

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documented effects are generally lacking. Carr (1983) inferred that "dredging and spoil disposal changed the configuration of the (Apalachicola) river" in such a way that Gulf sturgeon (*A. oxyrhynchus desotoi*) habitats were lost or modified. The U.S. Fish and Wildlife Service and Gulf States Marine Fisheries Commission (USFWS/GSMFC) (1995) prepared a Gulf sturgeon restoration plan, which mentions unpublished accounts of loss of deep hole habitat. Downstream movement of dredged material from a within-bank disposal site was reportedly responsible for the loss of habitat. Hastings' (1983) assessment of maintenance dredging activities in the Delaware River included the speculation that, although sediments resuspended during dredging would probably have little effect on adult shortnose sturgeon, sedimentation on a spawning ground could have severe consequences for developing eggs and larvae. He also speculated that resuspension of contaminated sediments would be deleterious to shortnose sturgeon. Moser and Ross (1995) tracked movements of both Atlantic (*A. oxyrhynchus*) and shortnose sturgeon through sections of the Cape Fear River, North Carolina, during dredging operations. Their data indicated a preferential sturgeon occupation of deep, midchannel areas, although no effects of dredging were detected. They speculated that a tendency for sturgeon to move in the upper portion of the water column might reduce overall risk of entrainment.

Shortnose and Atlantic sturgeon are among the most studied of all sturgeon species in terms of habitat and life history requirements (Hoff 1979; Dadswell et al. 1984; Van Den Avyle 1984; Gilbert 1989). Nonetheless, even for these species the lack of basic fishery resource data specific to given river and estuarine systems remains a significant challenge to balancing sturgeon protection and dredging project management needs effectively . While overall patterns of seasonal movements are known (e.g., McCleave, Fried, and Towt 1977; Brundage and Meadows 1981; Buckley and Kynard 1981; Hastings et al. 1987; Hall, Smith, and Lamprecht 1991; Moser and Ross 1995; Kieffer and Kynard 1983; O'Herron, Able, and Hastings 1983), timing of sturgeon occurrences in specific reaches of navigable waterways can vary substantially from year to year. This general finding of inadequate distribution and habitat requirement data pertains to stocks other than Atlantic and shortnose sturgeon, including white sturgeon and Gulf sturgeon (Parsley, Beckman, and McCabe 1993; USFWS/GSMFC 1995). Inflexible dredging project contract schedules cannot easily accommodate such unpredictability.

Both the acknowledged need to restore sturgeon stocks (Birstein 1993; Waldman and Wirgin 1998) and the need to maintain navigable waterways justify the development of tools to address gaps in the state of knowledge required to protect these resources. For example, biochemical determination of sturgeon presence/absence could be used to focus conventional fisheries sampling methods more effectively.

CONCEPTUAL APPROACH: An ideal tool that would satisfy these objectives would be capable of (1) detecting the presence of sturgeon within a finite volume of water with a high degree of confidence, (2) identifying sturgeon biochemical "signatures" with resolution to the species level, and (3) estimating relative density of the source sturgeon stock. Ultimately, it may be feasible to discern life history stage, sex, and reproductive condition from biochemical cues. In addition, the tool should be cost-effective and should not require extensive, time-consuming laboratory analyses. Biochemical approaches were selected after consideration of other sensing modalities, including underwater acoustics. Although promising in certain respects, acoustics, i.e., listening for sturgeon

sounds in a fashion analogous to songbird surveys, appears most feasible for detection of spawning congregations rather than broad area surveys.

Development of a prototype tool for sturgeon detection has focused to date on satisfying the first objective, simply a capability to confirm the presence or absence of sturgeon biochemical cues in a given water sample. Technical challenges confronting this stage of tool development include screening and identification of chemical compounds unique to sturgeon, potentially at the species taxonomic level, and determination of the properties of these compounds such that optimal sample processing procedures can be devised. Initial investigations, conducted in Russia and at the U.S. Geological Survey's Conte Anadromous Fish Research Center (CAFRC) in Turners Falls, Massachusetts, have concentrated on determining candidate compounds. These efforts are summarized in the following paragraph.

The search for suitable "sturgeon signature" compounds has progressed along several lines. Several categories of exometabolites, substances released by individual fish into the water column, are potentially detectable. All aquatic organisms discharge into their surroundings picograms or lesser quantities of chemical substances that potentially contain biological information. Examples include feces, urine, bile, sperm or ovarian fluids, and mucus (Salin and Williot 1991). Sturgeon have highly developed olfactory senses, linked to their migratory and foraging behaviors, and it is not inconceivable that chemical cues such as these play an important role in intra-specific communication (Doeving 1986). In principle, therefore, physicochemical detection is analogous to evolved olfactory mechanisms employed by many fish species (Kasumyan 1993, 1996).

TECHNICAL APPROACH: The basic approach followed in this technical note involves the following:

- Collection of water samples from the waterway for which sturgeon presence/absence is to be determined.
- Concentration of exometabolites in a given sample by 5-6 orders of magnitude using Solid-Phase Extraction (SPE) techniques.
- Thin-Layer Chromatography (TLC) analysis of accumulated extracts of sturgeon exometabolites.

Determination of Candidate Exometabolites

Moscow experiments. An initial step in this investigation was screening of candidate "marker" substances. In December 1996 and February 1997, samples of mucus, bile, fecal wastes, urine, and sperm and ovarian fluid were obtained from immature and adult male and female specimens of Siberian sturgeon (*A. baerii*) at a Russian fish processing factory on the Volga River. Lipid substances were extracted from bile gland secretions and from the lower digestive tracts of 18 sturgeon specimens. Human bile was used as a comparative control because its chemical composition is well known. Initial attempts at discrimination involved lyophilic drying methods and standard TLC procedures. Samples were analyzed for the presence of free and linked amino acids. In addition, carbohydrate and polysaccharide contents were determined. Results of these specific analyses were unproductive, however, insofar as their use as biochemical markers was

concerned. Concurrently, successful recovery of candidate substances in quantities of approximately one nanogram per liter on silica gels indicated that derivatives of bile acids represented promising markers. Subsequently, alkaline hydrolysis was used to establish that Siberian sturgeon bile acids were composed largely of deoxycholic acid with cholic and lithocholic acids. Chromatographic retention times for these substances were determined relative to standards.

As a crude preliminary test of taxonomic sensitivity, samples of human, cattle, rainbow trout, salmon, herring, and sturgeon bile secretions were subjected to these analyses. Albeit for grossly disparate taxonomic groups, specificity in terms of the ratios of deoxycholic, cholic, and lithocholic acids was readily displayed. Based on these encouraging results, bile acids became the focus of further investigation. Bile acids derived from Siberian sturgeon were next compared with those of sterlet (*A. ruthenus*). However, the ratios of the three major component acids proved to be so similar that species-level discrimination was unlikely based on these data alone. Therefore, in the search for other species-specific chemical markers, concentration procedures that would enhance sensitivity of the tests became a high priority.

Development of an effective laboratory procedure for analysis of odorant components of exometabolites. Laboratory experiments conducted in Moscow attempted to differentiate water samples obtained from aquaria housing various fish species, including Siberian sturgeon, Amur sheatfish (*Parasiluris asotus*), and South American knifefish (*Apteronotus albifrons*). Odorants were collected by floating 300-cm^2 squares of ultrathin (10 micron), specially prepared, water-resistant quartz (inorganic, chemically inert, lipophilic) fiber cloth at the water/air interface. In one experiment, water samples were withdrawn from a 50-liter aquarium holding a single Siberian sturgeon. The sturgeon was not fed while in the aquarium. Samples (500 ml) were collected 24, 72, and 144 hours after placement of the sturgeon in the aquarium. A 100-ml subsample was subjected to an SPE process using Supelco C8 extraction tubes. Tube contents were extracted using 5 ml of methanol followed by 5 ml of chloroform. Resulting solutions were evaporated and the residues dissolved in 10 µl of chloroform. Of this solution 1 µl was painted on a Kieselgel-60 TLC plate, which basically involves plotting of processed concentrates onto the plate via capillary action.

The concentrated substances were separated using adsorption (chloroform; chloroform:methanol, 9:1 or 1:1; methanol) and distribution (chloroform:methanol:water, 61:32:7, etc.) chromatography procedures in a TLC chamber. Comparisons could then be made of unknowns versus standards, which in this case represented both free and conjugated bile acids, with respect to their retention times (Rf). The presence of compounds outside the visible light range was checked by treatment with 5 percent sulfuric acid in ethanol and application of heat. Five distinct compounds on a strong background were observed. Background strength increased through the samples representing the longer duration experiments.

CAFRC experiments. Similar laboratory studies were undertaken at the CAFRC. U.S. Geological Survey personnel at this facility have been engaged in research into sturgeon population dynamics and management for several decades. In particular, the Center has interests in both shortnose and Atlantic sturgeon, which inhabit the nearby Connecticut River system.

The Center maintains tanks containing multiple life history stages of several sturgeon species, as well as other anadromous species, thereby providing ready access to water samples for exometabolite screening. Holding tanks are approximately 2 m in diameter and 1 m in height, containing fish maintained separately in tap or river water. Series of water samples (500-1,000 ml) were collected in glass vessels from the holding tanks. One vessel series contained tap water as a control (vessel 1) for comparison with samples (vessel 3) taken from a holding tank with shortnose sturgeon. Another vessel series contained river water as a control (vessel 2) for comparison with samples taken from tanks holding Atlantic sturgeon (vessel 4) and Atlantic salmon (*Salmo salar*, vessel 5), respectively.

TLC analyses of the water samples representing the five treatments were undertaken using standard methods (Touchstone 1992). TLC results could be observed directly in the visible light range without a need for enhanced visualization methods, e.g., viewing in ultraviolet light. The ensuing SPE process used SPE tubes packed with 500 mg of appropriate sorbents (LC-8, LC-18, LC-SAX, LC-SCX, ENVI-C and others) applied to 100- to 1,000-ml samples of water. A total of over 300 SPEs on 150 water samples was conducted. Optimal results in terms of reproducibility and maximal fish exometabolite accumulation were obtained from the most hydrophobic sorbents (LC-8 and LC-18).

For liquid extraction a Sigma Visiprep-DL SPE manifold was employed with various High Performance Liquid Chromatography (HPLC) grade solvents, including hexane, chloroform, and methanol. Adsorbed substances were washed with 5 ml of methanol and chloroform or hexane, and the extracts evaporated to a volume of 5 µl. Then 0.1-1.0 µl of the solution was transferred to a TLC plate (Kieselgel 60 or 60 F254) and developed by adsorption chromatography according to the increasing polarity of solvents (hexane-methanol) or combinations of solvents of neutral pH (chloroform:methanol:water, 61:32:7) or acidic pH (toluene:acetic acid:water, 50:50:10; upper phase, butanol:acetic acid:water, 10:1:1; chloroform:acetone:acetic acid, 7:2:1 or 5:2:3).

SPE of substances contained in the shortnose and Atlantic sturgeon-exposed water samples was facilitated by using quartz fiber substrates as described for the initial Moscow experiments.

Visualization of the components on TLC plates involves application of a 5 percent solution of sulfuric acid in ethanol with heat. Alternatively, solutions of concentrated sulfuric acid, acetic anhydride-sulfuric acid, or phosphornomolybdenic acid were used. Figure 1 exemplifies three chromatograms obtained by these methods for samples taken during the CAFRC experiments, illustrating movement of substances on the chromatography plates as a consequence of Rf. Distinct patterns of substance separation evidenced by the magnitude of Rf-driven movement on the TLC plates are apparent. The first two chromatograms, respectively, represent samples developed in the UV-range (254 and 365 nm) using methanol as an eluent and without enhancement, showing detection of organic substances for Atlantic salmon but not for Atlantic sturgeon. The third chromatogram was obtained using acetone as an eluent followed by treatment with 5 percent sulfuric acid in ethanol and application of heat. Pattern development was more distinct when illuminated under UV light. Clear differences were observed in patterns of organic substance separation for the two sturgeon species (*A. brevirostrum* and *A. oxyrhyncus*) as well as Atlantic salmon (*S. salar*). Separation in acetone yielded three chromatographic spots for shortnose sturgeon, three for Atlantic sturgeon, and five for Atlantic salmon.

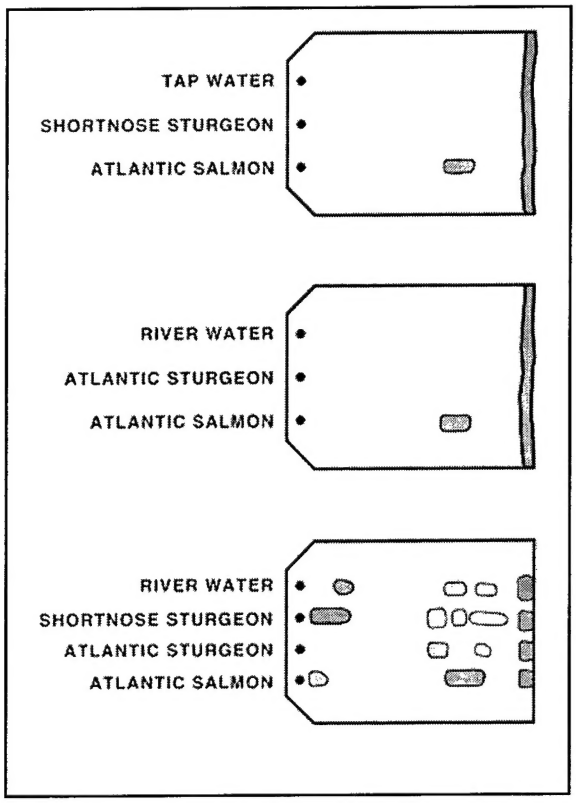

Figure 1. Examples of chromatograms derived by various techniques (see text). Separation of substances occurs in the vertical plane above concentrate sample points representing tap water, river water, shortnose sturgeon exometabolite, Atlantic sturgeon exometabolite, or Atlantic salmon exometabolite

The present interpretation of discriminated substances is that they represent free and unconjugated bile acids, including cholic acid, deoxycholic acid, glycocholic acid and its sodium salt, and sodium salts of taurochenodeoxycholic acid, taurocholic acid, and glycochenodeoxycholic acid. Threshold detection level is 100 ng/ml.

Technology Refinement. Preliminary results demonstrate that a capability to detect the presence of sturgeon (or other fish species) using biochemical methods is feasible. Further effort is needed to refine the methodology in order to satisfy requirements for a reliable field data acquisition capability. Analytical studies are underway to confirm identities, sources, and characteristics of the substances composing the sturgeon signatures or markers. Improvements to the chemical marker accumulation technique are being devised such that detection thresholds are lowered by an additional 1-3 orders of magnitude. Additional experiments are being pursued to explore the relationship between concentrations of chemical markers and density or biomass of sturgeon within a finite volume of water, and changes in concentration through time of exposure of sturgeon to a finite volume of water. At present, a single analysis requires from 0.1 to 1.0 L of extract and about three hours of laboratory processing. In the short term, expectations are to reduce sample processing time to less than 1 hour. It is believed that ultimately, real-time processing and marker detection and discrimination are possible.

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This technical note was prepared by Dr. Stanley Ross, Marine Physics Corporation; Dr. Edward Zinkevich and Ms. Varvara Vasileva, Severtsov Institute of Ecology and Evolution at the Russian Academy of Sciences, Moscow; Dr. Alexander Kasumyan, Moscow University Biology Faculty; and Dr. Douglas Clarke, Environmental Laboratory, Vicksburg, MS, U.S. Army Engineer Research and Development Center.

POINTS OF CONTACT: For additional information, contact Dr. Clarke (601-634-3770, *Douglas.G.Clarke@erdc.usace.army.mil*) or the Program Manager of the Dredging Operations and Environmental Research Program, Dr. Robert M. Engler (601-634-3624, *Robert.M.Engler@erdc.usace.army.mil*). This technical note should be cited as follows:

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